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TI Ablating the ischemia-reperfusion injury in non-heart-beating donor
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AU Hernandez A; Light J A; Barhyte D Y; Mabudian M; Gage F
definition of Belzer MPS
SO TRANSPLANTATION, (1999 Jan 27) 67 (2) 200-6.

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AN 1998302706 MEDLINE
DN 98302706 PubMed ID: 9638850
TI Optimal pH for simple cold storage or machine perfusion of dog kidneys
with UW solution.
AU Lindell S; Nobel M; Rankin M; D'Alessandro A; Southard J H
SO TRANSPLANT INTERNATIONAL, (1998) 11 (3) 208-11.

AN 95291541 MEDLINE
DN 95291541 PubMed ID: 7773485
TI Prostaglandin E1 attenuation of ischemic renal
reperfusion injury in the rat.
concentration of PGE1
AU Vargas A V; Krishnamurthi V; Masih R; Robinson A V; Schulak J A
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ABLATING THE ISCHEMIA-REPERFUSION INJURY IN NON-HEART-BEATING DONOR KIDNEYS^{1,2}

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Background. The objective of this study was to determine if allopurinol (AL) and/or trifluoperazine (TFP) added to the Belzer machine preservation solution (MPS) improves the function of non-heart-beating donor (NHBD) canine kidneys.

Methods. Anesthetized canines underwent bilateral dissection of the renal vessels, obtaining baseline flow. After removing one kidney (heart-beating donor [HBD]), the dog was exsanguinated. After remaining in situ for 120 min (30-min warm ischemia time, 90-min cold ischemia time), the second kidney was removed (NHBD), flushed, biopsied, and weighed. The kidneys were machine-perfused separately for 20 hr, and pressure, flow, and resistance were measured serially. The kidneys were randomly assigned to a perfusate group (G): G1=MPS, G2=MPS+TFP, G3=MPS+AL, and G4=MPS+TFP+AL. Kidneys were implanted separately into a single recipient dog. Flow, resistance, and urine output were measured serially for 4 hr. Blood and urine samples and kidney biopsies were then obtained. All measurements were standardized to 100 g of kidney weight.

Results. HBD kidneys functioned better than NHBD kidneys in all groups, as expected. Although perfusate G1 was the most effective solution for HBD kidneys, the TFP additive (perfusate G2) more effectively reversed the vasospastic effects of ischemia/reperfusion for NHBD than the MPS solution (G1) with or without other additives. In HBD kidneys, the addition of AL resulted in the best creatinine clearance; however, AL was less effective than MPS alone in NHBD kidneys. TFP+AL together were completely ineffective in pre-

serving renal function, regardless of whether the kidneys were from HBD or NHBD.

Conclusions. MPS+TFP more effectively protected renal function against reperfusion injury in the NHBD than MPS alone, AL, or AL+TFP. AL exerts a salutary effect on creatinine clearance in HBD but not in the NHBD. The TFP and AL combination should not be used together with the MPS in machine preservation of kidneys.

The progressive lengthening of the waiting list and the failure of kidney donation to keep pace relative to the size of the list have led to the expansion of the criteria for organ donation, including renewed interest in the usage of kidneys from non-heart-beating donors (NHBD*) (1, 2). With current preservation solutions, there is an unacceptably high rate of delayed graft function (DGF) and primary nonfunction (PNF), indicating inadequate protection against the obligatory injury (DGF: 67-76%; PNF: 6-8%). Furthermore there is also accumulating evidence that DGF may not only predispose to acute rejection but also have an independent deleterious effect on long-term graft survival (3-6).

The presence of prolonged warm ischemia time (WIT) is the main difference between heart-beating donors (HBD) and NHBD. We believe that organs from the NHBD may require specially designed preservation solutions with capabilities of blocking key processes involved in the ischemia/reperfusion (I/R) response. Current preservation solutions were designed

* Abbreviations: AL, allopurinol; AUC, area under the curve; CCr, creatinine clearance; CI, confidence interval; CIT, cold ischemia time; DGF, delayed graft function; G1, group 1; G2, group 2; G3, group 3; G4, group 4; HBD, heart-beating donor; I/R, ischemia/reperfusion; MAP, mean arterial pressure; MPS, Belzer machine preservation solution; NHBD, non-heart-beating donor; PNF, primary nonfunction; RBF, renal blood flow; ROS, reactive oxygen species; RVR, renal vascular resistance; TFP, trifluoperazine; WIT, warm ischemia time; XD, xanthine dehydrogenase; XO, xanthine oxidase.

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for organs from the HBD and do not meet this requirement, as evidenced by the 2–3-fold higher rate of DGF in NHBD compared to HBD.

We believe that using enhanced preservation solutions during the pulsatile preservation period may improve the kidney's ability to tolerate the reperfusion response and thereby improve the immediate function rate of NHBD kidneys. If this *ex vivo* resuscitation is effective in improving the quality of renal function, then interest may be stimulated to expand the utilization of organs from the NHBD, an underutilized resource presently.

After ischemia, intracellular ATP rapidly decreases and ADP increases temporarily. Catabolism of ADP leads to the formation of adenosine, inosine, and hypoxanthine. Loss of ATP disrupts the calcium gradient with increased levels of intracellular calcium, and activating the calcium dependent proteases that convert xanthine dehydrogenase (XD) to xanthine oxidase (XO) (7). As XO uses oxygen for the conversion from hypoxanthine to xanthine to uric acid, XD is depleted, which leads to accumulation of hypoxanthine during the ischemic period.

After reperfusion, the oxidation of the hypoxanthine and xanthine generate reactive oxygen species (ROS). Although ROS are generated during other metabolic processes, hypoxanthine oxidation is the main source of ROS during reperfusion (8). ROS causes lipid peroxidation, cell membrane damage, direct oxidation of membrane proteins, and DNA damage. This results in increased cell membrane permeability and enzymatic disturbances.

Allopurinol (AL), a competitive xanthine oxidase inhibitor, has been shown in previous studies to decrease I/R injury (9–11) by reducing ROS formation during reperfusion. It also enhances the nucleotide pool by preventing catabolic destruction of the purines (12). There is also evidence that AL may have beneficial effects on antioxidant defenses against I/R injury in rat kidneys by enhancing glutathione levels (13).

The calmodulin-Ca complex when activated regulates enzymatic processes involved in membrane phosphorylation, microtubule disassembly, release of neurotransmitters, and activation of phospholipase A₂. During ischemia, the calmodulin-Ca complex activates phospholipase A₂, leading to inner membrane mitochondrial dysfunction. Trifluoperazine (TFP) inhibits the calmodulin-Ca complex, and theoretically it will block all these deleterious enzymatic reactions (14).

In cultured smooth muscle cells, Anderson et al. (15) reported that the binding of calcium to one site on calmodulin activates phosphodiesterase, while the binding of calcium to all four sites activates myosin light chain kinase, which produces vasoconstriction, resulting in decreased renal blood flow (RBF). The addition of TFP to preservation solution has resulted in increased survival rate of cold storage dog kidneys (16, 17). It has been suggested that TFP added to the perfusate exerts a protective effect upon the microcirculation without reducing vascular resistance (16). The purpose of this study, therefore, was to determine if adding AL and/or TFP to the current preservation solution (MPS) will improve the immediate function of ischemically damaged kidneys from NHBD.

MATERIALS AND METHODS

The experiment was performed using 21 adult female, conditioned, tricolored mongrel, non-pregnant, non-lactating dogs, weigh-

ing 45–50 lb. Dogs were fasted for 24 hr before the experiment but allowed to have water. General anesthesia was induced by intravenous pentobarbital (25 mg/kg) and maintained with halothane in oxygen as needed. Dogs were intubated and ventilated with a respirator at the rate of 15 cycles/min with a tidal volume of 20 ml/kg body weight. Normal saline was administered intravenously throughout the procedure as needed to maintain a mean arterial pressure (MAP) of 90–100 mmHg. An arterial line was placed through the left internal iliac artery, and the tip of the catheter was advanced to the level of the renal arteries. The dogs' temperature was maintained by using a warming/cooling blanket.

Animal Surgery

Baseline flow measurements. The donor dog underwent bilateral dissection of the renal vessels. After 10 min, flow probes were placed on the renal arteries and blood flow (RBF) and MAP were measured simultaneously in both kidneys. The renal vascular resistance (RVR) was calculated using the following equation: $MAP / \text{mean RBF}$. Right and left kidneys were randomly assigned to the HBD group, and the first kidney (HBD) was removed, weighed, flushed, biopsied, and placed on machine preservation with the test perfusate. The dog was then exsanguinated by a right atrial cannula and suction. After the heart stopped (NHBD), the cannula was advanced into the inferior vena cava to drain the perfusate during the *in situ* preservation portion. A second cannula was inserted into the thoracic aorta and advanced at the level of the renal arteries for *in situ* flushing. The dog was then closed for 30 min (the warm ischemia period), after which *in situ* preservation was carried out for 90 min with combined intravascular and intraperitoneal cooling. The average temperature reached during the cooling period was 10.356°C (range: 2.4–18). The second kidney (NHBD) was then removed, weighed, flushed, biopsied, and placed on machine preservation with the same solution but in a different cassette from the HBD mate kidney. This canine model of shock and blood loss closely mimics the human trauma victim who dies from hypovolemia and its resultant reduced RBF. The solutions were randomly assigned as follows: group 1 (G1), n=12: Belzer machine preservation solution (MPS) (control group); group 2 (G2), n=10: MPS + 0.2 mg of TFP/ml; group 3 (G3), n=10: MPS + 0.4 mg of AL/ml; group 4 (G4), n=10: MPS + 0.2 mg of TFP + 0.4 mg of AL/ml.

Pulsatile preservation period. Pulsatile preservation was used for all kidneys. Each kidney was perfused for 20 hr at 4°C in a separate cassette. The systolic pressure was maintained at 40 mmHg for 4 hr and then allowed to autoregulate. The pressure, flow, and resistance were measured at the beginning, repeated hourly for 3 hr, and again at the end of the pump preservation period.

Implantation of the HBD and NHBD kidneys. Both kidneys were randomly implanted the next day on the same dog. The external iliac artery and vein were dissected bilaterally, and end-to-end anastomoses were performed to the renal vessels. Dogs were hydrated with normal saline, and 12.5 g of mannitol were infused before each reperfusion. MAP and flow were measured initially and then hourly for 4 hr in each kidney. Urine was collected separately from each ureter in a graduated cylinder. Blood samples and kidney biopsies were obtained at the end of the 4-hr observation period. The biopsies were snap-frozen for future study.

Blood Flow, MAP, and Resistance Measurements

Blood flow was measured with a perivascular transonic flow probe (Transonic Medical Volume Flowmeter, model HT207) (18). Systolic and diastolic flows were recorded, and the mean RBF was calculated using the following formula: $RBF = (SF + 2DF)/3$. The MAP was continuously recorded, and RVR was calculated using the following formula: $RVR = MAP/RBF$. The area under the curve (AUC) for flow and resistance was calculated for the 4-hr observation period according to Tai's mathematical model (19).

Measurements of Renal Function

Total urine output, urinary sodium and potassium, and serum and urine creatinine were measured, and Na/K ratios and creatinine clearance (CCr) were calculated for each kidney and standardized to 100 g of kidney weight.

Statistics

Statistical analysis was done with SAS software. Analysis of covariance models were used to compare the mean values adjusting for kidney type, perfusate, and their interaction. NHBD and HBD kidney types were compared, adjusting for perfusate groups. *P*-values less than 0.05 were considered statistically significant. AUC were calculated for flow and resistance over time. Results are expressed as mean \pm SEM or 95% confidence intervals (CI).

RESULTS

Baseline hemodynamics. Before the organ recovery, mean baseline flow and resistance for the kidneys still in situ were 365 ± 35 ml/min and 0.223 (0.201 – 0.264 , 95% CI) mmHg/(ml/min), respectively ($n=36$ kidneys).

Pump preservation. During pump preservation, NHBD kidneys had inferior hemodynamic parameters to HBD, as measured by mean flow and mean resistance for the combined groups (77 ± 7 vs. 120 ± 7 ml/min, $P=0.0002$ and 0.542 (0.425 – 0.693 , 95% CI) vs. 0.308 (0.243 – 0.391 , 95% CI), $P=0.001$). The AUCs for flow and resistance during pump preservation also accurately reflect the added ischemic injury of the NHBD vs. the HBD (304 ± 38 vs. 508 ± 33 mmHg/(ml/min)/4 hr, $P=0.006$, and 0.093 (0.024 – 0.358 95% CI) vs. 0.006 (0.002 – 0.018 95% CI) mmHg/(ml/min)/4 hr, $P=0.0026$ (Table 1). However; there was no difference in the AUC for flow and resistance between G1 and G2 (515 ± 51 and 516 ± 47 mmHg/(ml/min) for 4 hr, $P=0.98$) and (0.004 and 0.003 , $P=0.93$). Thus the superior performance of kidneys preserved with the TFP additive must depend on other preservation properties rather than on its direct vasodilating action (Table 1).

Reperfusion hemodynamics. After 15–30 min following reperfusion, RBF decreases were followed by varied degrees of spontaneous recovery, presenting different patterns of flow during reperfusion among different perfusate groups (Fig. 1).

On reperfusion, initial RBF was higher in HBD than NHBD regardless of the perfusate group; however, among the perfusates, solution G2 had the highest mean initial RBF measurements for both NHBD and HBD (Fig. 2). Means adjusted for kidney type demonstrated that G2 was superior to G1, G3, and G4 in preserving hemodynamics as measured

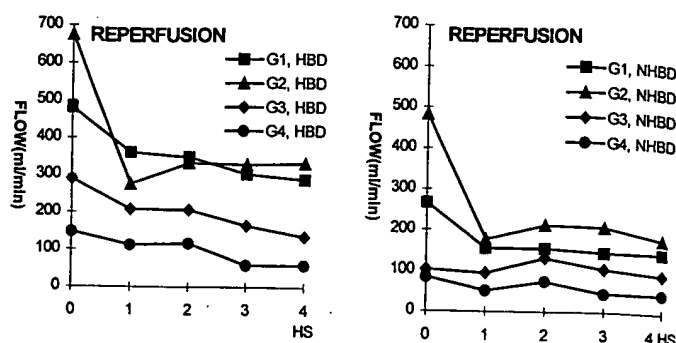


FIGURE 1. RBF during reperfusion and 0, 1, 2, 3, and 4 hr afterwards. Data were standardized to 100 g of kidney weight. The graph on the left shows the HBD by perfusate group; the graph on the right shows NHBD by perfusate group.

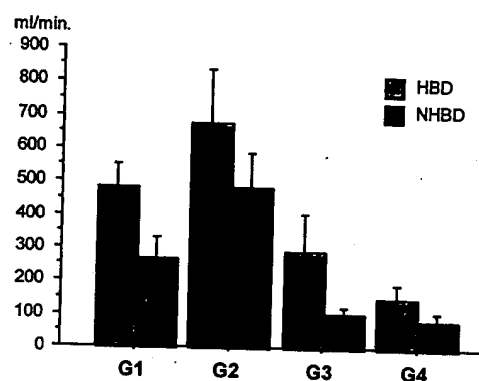


FIGURE 2. Initial RBF on reperfusion. The data were standardized to 100 g of kidney weight and are presented as mean \pm SEM.

by the initial RBF on reperfusion (578 ± 68 vs. 373 ± 53 , 195 ± 53 , and 116 ± 84 ml/min, $P=0.03$, 0.0002 , and 0.0003 , respectively) (Table 2). In fact, NHBD-G2 kidneys function as well as HBD-G1 when function is measured by the initial reperfusion RBF (482 ± 103 vs. 481 ± 70 ml/min, $P=0.992$) (Fig. 2). Furthermore, the RVR on initial reperfusion measurements were similar for NHBD-G2 and HBD-G1 (0.197 (0.099 – 0.789 , 95% CI), and 0.203 (0.083 – 0.494 , 95% CI), $P=0.937$). Mean RBF during the entire reperfusion period (mean values over 4 hr) was also better in the kidneys preserved with the G2 solution than when G1, G3, or G4 were used. (318 ± 32 vs. 269 ± 30 [$P=0.275$], 150 ± 26 [$P=0.0006$], and 79 ± 12 [$P=0.0001$], respectively). The decrement in flow

TABLE 1. Area under the curve for flow and resistance during pump preservation^a

Perfusate	Pump flow ((ml/min)/4 hr)				Pump resistance (mmHg/(ml/min)/4 hr)			
	AUC				AUC			
	N	AUC	SE	P	N	AUC	95% CI	P
G1 (control)	7	515	51		7	0.004	0.0006–0.03	
G2	8	516	47	0.989	8	0.0038	0.0006–0.02	0.934
G3	6	302	54	0.008	6	0.102	0.01–0.9	0.012
G4	7	229	50	0.004	7	0.190	0.02–1.3	0.002
Kidney type								
HBD	16	507.6	33		16	0.006	0.002–0.018	
NHBD	12	304.1	38	0.0006	12	0.093	0.024–0.358	0.002

^a The data were standardized to 100 g of kidney weight and adjusted by kidney type and perfusate group.

TABLE 2. Initial reperfusion RBF and resistance^a

	Initial RBF (ml/min)				Initial RVR mmHg/(ml/min)			
	N	Mean	SE	P	N	Mean	95% CI	P
Perfusate								
G1 (control)	10	373	53	0.026	8	0.262	0.167–0.410	0.212
G2	6	578	68		6	0.180	0.103–0.316	
G3	10	195	53	0.0002	9	0.742	0.492–1.122	0.0001
G4	4	116	84	0.0002	4	0.907	0.392–2.100	0.0001
Kidney type								
HBD	15	406	45		14	0.326	0.237–0.447	
NHBD	15	225	45	0.006	13	0.549	0.395–0.763	0.019

^a The data were standardized to 100 g of kidney weight and adjusted by kidney type and perfusate group. Mean values are compared against G2, and HBD versus NHBD.

TABLE 3. AUC for flow and resistance during the 4-hr reperfusion period^a

	AUC							
	Reperfusion flow ((ml/min)/4 hr)				Reperfusion resistance (mmHg/(ml/min)/4 hr)			
	N	AUC	SE	P	N	AUC	95% CI	P
Perfusate								
G1 (control)	7	1026	117		7	0.024	0.005–0.108	
G2	6	1174	126	0.399	6	0.018	0.003–0.097	0.739
G3	9	596	103	0.012	8	0.547	0.142–2.114	0.001
G4	4	312	154	0.001	4	6.668	0.523–84.9	0.0001
Kidney type								
HBD	13	994	88		13	0.073	0.027–0.197	
NHBD	13	560	87	0.001	12	0.553	0.197–1.555	0.005

^a The data were standardized to 100 g of kidney weight and adjusted by kidney type and perfusate group.

TABLE 4. Mean values of measurements for kidney function among different perfusate groups and kidney types^a

	Urine output (ml)				Na/K ratios				CCr (ml/min)			
	N	Mean	95% CI	P	N	Mean	95% CI	P	N	Mean	95% CI	P
Perfusate												
G1 (control)	11	44.4	20.1–97.9		9	3.0	1.8–5.0		9	0.92	0.35–2.4	
G2	9	45.3	18.8–109	0.9694	8	2.6	1.6–4.4	0.6917	7	1.02	0.3–3.0	0.8758
G3	10	28.6	12.6–64.8	0.4007	8	3.5	2.0–5.8	0.6518	6	0.6	0.16–2.1	0.5309
G4	6	4.0	1.2–13.4	0.0004	5	7.1	3.2–15.6	0.0295	2	0.04	NA	0.0062
Kidney type												
HBD	19	31.3	17.5–55.1		16	2.16	1.55–3.01		13	1.28	4.03–22.16	
NHBD	17	15.3	8.4–27.8	0.0775	14	6.61	4.56–9.56	0.0001	11	2.47	1.15–5.34	0.021

^a The data were standardized to 100 g of kidney weight and adjusted by kidney type, perfusate group, and dog weight. The mean values of different perfusate groups are compared to G1, and the mean values of NHBD are compared to HBD. Kidneys that did not produce urine on reperfusion were excluded from the mean urine output calculation. Four kidneys did not produce urine—one in each of the following groups: G1-NHBD=1, G2-NHBD=1, G4-NHBD=1, and G4-HBD=1.

during reperfusion was also less in G2 kidneys than in G1, G3, and G4 (13% vs. 26%, 59%, and 78%, respectively). These results, taken in concert, show that TFP added to MPS improves function as measured by flow and resistance in the ischemic kidney to values similar to the nonischemic kidney despite the added 120 min of combined warm and cold ischemia.

During reperfusion, the AUC for flow in G2 was higher than G1 (1174 ± 126 vs. 1026 ± 117 ml/min for 4-hr reperfusion period, $P=0.39$). G3 and G4 AUCs were 42% and 70% lower than the control group, respectively (Table 3).

Despite the above observations, during reperfusion, the combined AUCs for flows and resistances for the two major groups, NHBD and HBD, were significantly different from

each other (560 ± 87 vs. 994 ± 88 ml/min, and 0.553 ± 0.469 vs. 0.073 ± 0.457 mmHg/(ml/min)) ($P=0.002$ and 0.005 , respectively) (Table 3). This result indicates that the AUC for flows and resistances more accurately reflects reperfusion injury than simple measurements at a point in time.

Kidney function. Only four kidneys failed to produce urine during the 4-hr reperfusion period—one in each of the following groups: G1-NHBD, G2-NHBD, G4-HBD, and G4-NHBD. In G3-HBD and G3-NHBD, all grafts produced urine.

Adjusted values by perfusate group showed that all NHBD kidneys functioned less well than HBD kidneys regardless of the perfusate group (urine output: 15.3 vs. 31.3, $P=0.07$; Na/K ratios: 6.6 vs. 2.1, $P=0.0001$; and CCr: 2.5 vs. 1.3, $P=0.02$) (Table 4).

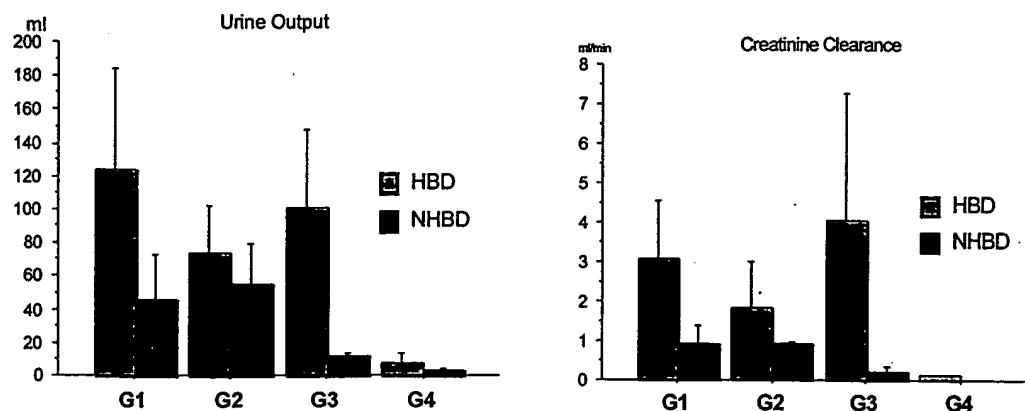


FIGURE 3. Urine output and CCr. Data were standardized per 100 g of kidney weight, and values are expressed as mean \pm SEM. Kidneys that did not produce urine on reperfusion were included for the mean urine output calculation. Four kidneys did not produce urine, one in each of the following groups: G1, NHBD; G2, NHBD; G4, NHBD; and G4, HBD.

AL in HBD (G3) resulted in the best CCr; however, this salutary effect disappeared in the NHBD kidneys. Similarly, the urine output was preserved by AL only in the HBD kidneys, and the difference was not statistically significant when compared to the control group (101 ± 46 ml vs. 123 ± 60 ml, $P=0.925$) (Fig. 3). When the means among groups were adjusted to kidney type, the salutary effect of AL was lost, indicating that AL is an ineffective additive in severely ischemic kidneys where nucleotide stores are likely fully depleted. Kidney function in G2 appeared to be better than in G1, G3, and G4 as measured by urine output, Na/K ratio, and CCr, again suggesting that adding TFP improved the quality of kidney preservation (Table 4). The observed differences did not reach statistical significance because of small numbers in each group. Paradoxically, TFP+AL together (G4) were completely ineffective regardless of ischemia status. This group showed the worst urine output, CCr, and Na/K ratios (Table 4).

DISCUSSION

The mechanisms responsible for injury and cell death after ischemia and reperfusion are controversial. Extensive literature reviews (8, 20–22) agree that ATP depletion, intracellular calcium accumulation, generation of ROS on reperfusion, and activation of phospholipases and proteases are important factors in the I/R genesis. These catabolic events are blunted by hypothermia, the basis for organ preservation. However, in the NHBD, warm ischemia is a given condition, and, hence, more severe organ damage occurs than in the HBD because of the uninhibited metabolic processes (23). Current preservation solutions were designed for HBD organs, not NHBD organs, and hence do not meet the added requirements produced by the warm ischemia injury. We were interested in determining whether adding substances to the perfusate known to interfere with the vasospasm of ischemia and the generation of XO-dependent ROS would improve the quality of preservation of the NHBD kidney. We observed that, 15–30 min after reperfusion, RBF decreases and the resistance increases, followed by varied degrees of spontaneous recovery, suggesting different quality of microvascular preservation and different capacities to recover from the reperfusion injury between perfusate groups. This is

demonstrated by different patterns of flows and resistances during reperfusion among the different perfusate groups and different hemodynamic patterns between HBD and NHBD. These observations were made possible by using the ultrasonic transit time flow probe described by Bretan et al. in 1997, which accurately measures RBF and offers a practical method for assessing the severity of acute reperfusion injury (24). We believe the initial hemodynamic parameters result from the effects of warm and cold ischemia on the microcirculation. Thereafter, the hemodynamics reflect the reperfusion injury, featuring up-regulation of various cytokines attracting polymorphonuclear and platelets, the generation of ROS, and arachidonic acid-derived catabolites (22, 24, 25).

TFP added to MPS protected against vascular injury in both HBD and NHBD, as measured by ex vivo preservation parameters and in vivo hemodynamics on reperfusion. TFP also improved tubular function as measured by urine Na/K ratios. TFP has a potential for usage during the pump preservation period to preserve hemodynamics and tubular function. TFP added to the MPS (G2) is an initial step in addressing the I/R response. It resulted in better preservation characteristics and better function on reperfusion, as denoted by higher initial mean RBF measurements, in both the HBD and NHBD.

Multiple independent mechanisms have been implicated to explain the beneficial effects of TFP: lipid membrane stabilization, direct antioxidant activity, and calmodulin inhibition (14, 16, 26). TFP inhibits calmodulin, thereby inactivating myosin light kinase, thus promoting vasodilatation and enhancing RBF. However, other studies have suggested that TFP also stabilizes membranes by inhibiting the rise in free intracellular calcium, which blunts the activation of proteases and phospholipases that lead to cell death (16, 17). Perhaps these properties explain TFP's salutary effect on the microcirculation in our model. During pump preservation, the AUC for flow and resistance were similar between G2 and the control group, but during the reperfusion period, the AUC for those parameters were improved by TFP (G2) compared to the control group (G1). Interestingly, the beneficial effects of TFP were most pronounced in the NHBD group, where the I/R is more severe. Our results suggest that the

salutary effects of TFP on preservation are best observed during early reperfusion. Further investigation is warranted in this area.

The capacity to neutralize oxidative stress produced by ROS generated on reperfusion also plays a critical role in limiting the reperfusion injury response. AL protects against the I/R response by limiting the degradation of nucleotide pools and the formation of ROS (12). In our experiments, preservation with solution G3 containing AL resulted in the best CCr and total urinary creatinine excretion in HBD. The presumed mechanism is the inhibition of XO, a key enzyme in the generation of ROS. During ischemia, the adenine nucleotide pool falls rapidly, resulting in the accumulation of hypoxanthine. During reperfusion, hypoxanthine is converted to xanthine by XO, producing ROS, which directly damages the microcirculatory system (27). Furthermore, hypoxanthine freely diffuses out of the cell, disabling the salvage pathway and limiting the cell's ability to replace depleted energy stores. Paradoxically, the beneficial effects of AL were not observed in NHBD kidneys, which performed much worse than the controls (G1). We theorize that the nucleotide pools were severely degraded in the NHBD, and that XO is needed to regenerate energy stores from the perfusate substrates. The equilibrium between preventing degeneration and stimulating regeneration probably determines the salutary or detrimental effect of AL added to the perfusate.

We expected that TFP plus AL would have a synergistic effect in preserving kidneys but in fact the opposite occurred. Any beneficial effects seen from either drug alone in this model was neutralized by the combination, with the results in G4 much worse than the other groups. The results were particularly poor in the NHBD. As mentioned earlier, AL competitively inhibits XO activity while TFP decreases the rate of conversion from XD to XO (28). We speculate that XO inhibition with a concurrent decrease in conversion from XD to XO would lead to severe restriction of enzyme activity and inability to rebuild cellular energy stores and defense mechanisms. Further research is needed to find other agents to add to TFP and to reduce the reperfusion injury.

Two other observations of interest were noted in the course of these experiments. The transonic flowmeter accurately reflected the severity of the reperfusion injury, as measured by hemodynamic parameters and CCr. The AUC method, when used to compare trends over the 3-hr reperfusion period, improved the ability to detect differences between the HBD and NHBD groups.

CONCLUSIONS

Reperfusion injury is an unavoidable event in transplantation. However, effective organ preservation preserves or restores the cellular defense mechanisms necessary to protect against organ dysfunction. This protection was best observed in the G2 kidneys preserved with TFP added to the MPS, where the usual reflow vasospasm seen between 15 and 30 min autoreversed, whereas this improvement was not seen in the ischemically damaged organs from the other NHBD groups. From these experiments, it is reasonable to consider adding TFP to the MPS as an initial step in developing a more effective solution for the ex vivo resuscitation of ischemically damaged organs from NHBDs. These experiments were not designed to test agents administered after

revascularization that might be effective against the I/R injury. Presumably, these treatments would be additive to any ex vivo resuscitation effort. If the quality of NHBD organ function can be improved, then this underutilized resource can be more fully developed as another means to address the organ shortage.

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TRANSPLANTATION

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INTRAGRAFT CYTOKINE EXPRESSION AND TOLERANCE INDUCTION IN RAT RENAL ALLOGRAFTS^{1,2}

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Background. Intraoperative cytokine expression was evaluated in a model of renal transplantation. ACI and Lewis rats were used as donors and recipients, respectively, for heterotopic renal transplantation.

Methods. Treated allograft rats (n=10) received a preoperative dose of rapamycin and cyclosporine, followed by 7 days of cyclosporine postoperatively. Isograft rats (n=5) and control allograft rats (n=4) received no immunosuppression. Sacrifice was performed after 120 days. Expression of interleukin (IL)-4, IL-10, and interferon- γ (IFN- γ) transcripts was determined with semiquantitative reverse transcriptase-polymerase chain reaction.

Results. All treated allograft rats had normal function with 50% histologic rejection. All isografts had normal function. IL-4 and IL-10 were in greater density in allografts with normal histology, whereas IFN- γ was only seen in allografts with cellular rejection. No

IL-10 was seen in isografts, but IL-4 was detected in 3/5 isografts.

Conclusions. We conclude that the lymphocyte population's elaboration of IL-4 and IL-10 is associated with tolerance, whereas the production of IFN- γ and absence of IL-4 is associated with histology suggestive of acute cellular rejection.

The rejection of allotransplanted organs is mediated predominantly by host T cells that are specifically activated by donor alloantigen, but allospecific cytotoxic T cells can reside within an allograft without causing rejection (1). Based partly on these observations, Mosmann proposed a subdivision of mouse CD4 helper T-cell (Th*) clones based on differences in their pattern of cytokine production (2). The Th-1 cells secrete interleukin (IL)-2 and interferon- γ (IFN- γ). The Th-2 subset produces IL-4, IL-5, and IL-10. Acute cellular rejection (ACR) seems to require the presence of Th-1 cytokines and tolerance may be a result of suppression of Th-1 activity (3-6). Evidence in human cadaveric renal transplants suggests that IL-2 and IFN- γ production is associated with intense mononuclear infiltrates and clinical ACR (7). It

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* Abbreviations: ACR, acute cellular rejection; bp, base pair(s); CsA, cyclosporine; IFN- γ , interferon- γ ; IL, interleukin; PCR, polymerase chain reaction; RAPA, rapamycin; RT, reverse transcriptase; Th, helper T.

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